

THE ANOMALOUS INFLUENCE OF SALINITY ON TEMPERATURE TOLERANCES OF SUMMER AND WINTER POPULATIONS OF THE COPEPOD *EURYTEMORA AFFINIS*

BRIAN P. BRADLEY

*Department of Biological Sciences, University of Maryland Baltimore County,
Catonsville, Maryland 21228*

One of the more important variables in the estuaries and other environments is temperature. In these environments, survival of organisms, especially the more passive forms, depends on their tolerance to daily and seasonal fluctuations in temperature.

Temperature tolerances are in turn modified by other variables such as salinity and dissolved oxygen. These three variables are seasonally related in the estuary. Dissolved oxygen is physically dependent on temperature and to some extent salinity.

Previous work on temperature effects on marine invertebrates is reviewed by Kinne (1963, 1964, 1967, 1970). General treatments appear in Remane and Shlieper (1971) and in Vernberg and Vernberg (1972). These works also include discussions of the modifying effects of other variables. The classic paper on interacting variables is by McLeese (1956), working with the lobster *Homarus americanus*, who found that temperature of acclimation, salinity and oxygen tension all influenced the upper lethal temperature.

The present paper reports on experiments which were designed to find a suitable assay for thermal tolerance in the calanoid copepod *Eurytemora affinis* (Poppe), to investigate the effect of salinity on temperature tolerance and to compare the tolerances of populations of *E. affinis* collected at different seasons.

METHODS

The cultures of *E. affinis* were raised in sterilized bay water using the method of Heinle (1969b). Mature males were used in all the experiments, although no sex differences in thermal tolerance were detected in preliminary studies.

Elevated temperatures were obtained using a thermostatically controlled heating-stirring unit in an aquarium in which shell vials containing the test animals were placed. Water temperature was carefully monitored and no temperature gradients were detected in the aquarium. Time lags in temperature in the vials were 34-90 seconds, depending on the external temperature. Low temperatures were obtained using a cold room (2° C) and the heating unit used if necessary.

High temperature tolerance in the initial experiments was measured as the temperature at which half the test animals became inactive. Temperature was raised slowly, one degree every five minutes. Low temperature tolerance was measured similarly.

In subsequent assays, copepods were tested individually and their times and temperatures of collapse and their times of recovery at room temperature noted.

TABLE I

Times to succumb (TS) and times to recover (TR) of individual copepods subjected to sequential temperature changes and their correlations.

Traits					Obs.	Mean $\pm$ S.E. Minutes	
1 = TS, slow increase 25–35° C					26	44.0 $\pm$ 0.8	
2 = TR at 25° C, from 35° C					26	12.3 $\pm$ 1.3	
3 = TS, slow decrease 25–3° C					10	36.1 $\pm$ 0.8	
4 = TR at 25° C, from 3° C					10	3.5 $\pm$ 0.5	
5a = TS, 34.5° C shock, no prior cold exposure					10	4.9 $\pm$ 0.7	
5b = TS, 34.5° C shock, with prior cold exposure					10	6.1 $\pm$ 0.6	
6a = TR at 34.5° C, no prior cold exposure					10	9.4 $\pm$ 2.4	
6b = TR at 34.5° C, with prior cold exposure					10	13.8 $\pm$ 4.0	

Correlations*	2	3	4	5a	5b	6a	6b
1	-.66	0.58	-.36	0.06	0.50	-.50	-.47
2		-.13	0.09	0.14	-.66	0.42	0.37
3			-.27		0.42		-.39
4					0.02		0.36
5a						-.65	
5b							-.39

* With 10 observations, correlations of 0.58 and higher are significant ($P < 0.05$).

In all the tests where temperature was raised gradually the upper limit of tolerance was around 35° C, regardless of test salinity and osmotic acclimation. Thus a more discriminating assay was needed.

Following the work of Battaglia (1967) on recovery from osmotic shock, the assay finally used was based on the time to succumb (TS) and time to recover (TR) from a temperature shock of 34.5° C (just below the critical temperature noted above). For this assay single individuals in 2 ml water in shell vials were placed in the aquarium kept at a constant 34.5° C, using the heating-stirring unit. The assay continued for 30 minutes. Those animals which had not recovered at the end of the assay were given a TR score of 30-TS minutes. The times to succumb and times to recover were combined in an index (30 + TS - TR) for ease of interpretation. This index and the alternatives to it are discussed further in the results section.

Salinity was measured using a refractometer, each measurement being checked by at least one other observer. Animals were acclimated to various salinities for 24 hours, except where noted.

RESULTS

Temperature tolerance measured by the succumb-recovery method

In the initial experiment, 26 animals were exposed to a gradually increasing temperature (to 35° C), half of those recovering (10) to gradually decreasing temperature (to 30° C) and all of them, subsequently, to a shock of 34.5° C. The times to succumb (TS) and recover (TR) were observed in all cases, recovery being at room temperature (25° C). The means and standard errors of the eight measurements and the correlations among them are shown in Table I.

TABLE II

Times to succumb (TS) and times to recover (TR) from temperature shock of two populations of E. affinis acclimated for 24 hours at four salinities.

Populations	Salinity ‰							
	3		9		12		15	
	Mar	Aug	Mar	Aug	Mar	Aug	Mar	Aug
TS minutes	3.1	4.2	3.5	4.3	2.8	6.0	5.3	13.3
TR minutes	18.9	13.2	8.3	5.9	12.6	4.7	3.1	1.6
Number of obs.	19	19	19	19	10	10	10	10

	F values and significances	
	TS	TR
Between populations	14.6**	11.1**
Between salinities	12.4**	20.6**
3 vs. 9, 12, 15‰	6.0*	52.3**
9 vs. 12, 15‰	13.0**	1.0†
12 vs. 15‰	18.3**	8.4**
Interaction	5.0**	1.1†

* $P < 0.05$

** $P < 0.01$

† Not significant

Standard deviations within subclass were 3.6 minutes (TS) and 6.9 minutes (TR)

As expected, animals resisting the shock for the longest period tended to recover earlier. The 12 correlations between the various TS and TR are negative, with two exceptions. The average correlation between TS and TR is -0.34 ; and between TS and the TR immediately following is -0.49 . The 4 correlations between times to succumb are all positive, averaging 0.39 , as are those between times to recover, averaging 0.31 . Thus there appears to be consistency, not only between times to succumb and recover but also between high and low temperature tolerances. At least there does not seem to be a negative correlation between tolerance to high and to low temperatures, as might have been expected. And finally, cold shock did not significantly affect subsequent tolerance to high temperature shock.

Strain differences influenced by salinity with acclimation

Descendents of animals collected in March 1973 from the Patuxent River (Maryland) were compared with progeny of a sample collected further upstream in August 1973. Tests were done in the salinities at the collection sites (6‰ for March and $<1‰$ for August) and also after acclimation in the other salinity. The critical upper temperature, when raised gradually, was slightly higher for August $0-1‰$ than for March 6‰ ($35.7, 34.4^{\circ}\text{C}$), and for August in 6‰ than for March in $0-1‰$ ($35.5, 34.1^{\circ}\text{C}$). Average times of recovery (at room temperature) were 5, 6, 9, and 15 minutes, respectively. Thus the August population

TABLE III

Times to succumb (TS) and times to recover (TR) from temperature shock of two populations of E. affinis at two salinities without acclimation.

Population	Salinity ‰			
	0-1		12	
	Mar	Aug	Mar	Aug
TS min	2.9	3.1	3.4	5.2
TR min	20.3	13.4	12.2	9.5
Number of obs.	10	10	10	10
		F values and significances		
		TS	TR	
Between populations		14.3**	2.4†	
Between salinities		24.1**	3.8†	
Interaction		9.1**	0.5†	

** $P < 0.01$

† Not significant

Standard deviations within subclass were 0.8 minutes (TS) and 9.7 minutes (TR)

seems more tolerant to high temperature at both salinities. And tolerance seems greater in the salinities in which the samples were originally collected. While there is a suggestion of a strain difference in these results, they imply again that temperature of inactivation is not a useful measure and also that the tests should be done on individuals.

Individuals from the two populations acclimated to four salinities were tested using the shock-recovery assay. Days and salinities are partially confounded in the analysis, but at any salinity the results were repeatable between days. The results are shown in Table II. Clearly there were strain (population) differences and salinity differences in both time to succumb (TS) and to recover (TR); and salinity enhanced the strain difference, especially in TS. According to the means and the analyses of variance the critical change in salinity was between 9 and 15‰ for TS and between 3 and the higher salinities for TR. Whether measured by TS or TR, raising the salinity from 12 to 15‰ greatly increased the tolerance of both populations. Note that the effect of salinity was more systematic on the August population than on the March population. Tests were not done at salinities higher than 15‰ since the animals became almost inactive, although their behavior at 15‰ seemed quite normal.

Some of the animals tested at 15‰ did not succumb during the 30 minute assay period. These were given arbitrary scores of 29 minutes and 1 minute for TS and TR, respectively. The assay at 15‰ was repeated with the same animals at a higher temperature (36° C). The means for TS were 4.2 (March) and 5.0 minutes (August) and for TR were 14.3 (March) and 3.0 minutes (August). The difference between populations in TS was not significant ($0.05 < P < 0.10$). The difference in TR was significant ($P < 0.01$).

TABLE IV

Relationships between time to succumb and time to recover in two populations of E. affinis with and without salinity acclimation.

With salinity acclimation (3, 9, 12, 15‰)					
	d.f.	Sum of Squares (TS)	Sum of Products	Sum of Squares (TR)	r
Populations/salinity	4	389.5	— 250.3	677.9	—0.49
Salinities	3	488.8	— 853.1	2904.4	—0.72
Within	108	1411.6	— 146.5	5076.7	—0.05
Total	115	2289.9	—1230.9	8659.0	—0.27
Without salinity acclimation (0–1 and 12‰)					
Populations/salinity	2	16.4	— 31.1	274.5	—0.46
Salinities	1	16.9	— 78.1	360.0	*
Within	36	23.8	— 13.9	3416.6	—0.04
Total	39	57.1	— 123.1	4051.1	—0.26

* With one degree of freedom the correlation is trivially —1.

Strain differences influenced by salinity without acclimation

When animals were tested at salinities of 0–1‰ and 12‰, without acclimation, the effects of salinity and population on TR were not significant (Table III). Salinity and population still had a significant effect on TS. The differences between populations were in the same direction but smaller than in the tests after salinity acclimation. The effect of salinity acclimation was greater in the August population than in the March population.

The relationship between TS and TR

As was the case in an earlier experiment (Table I) the correlations between times to succumb and times to recover tended to be negative. We can now consider in more detail the relationships between TS and TR in the experiments reported above. The sums of squares for TS and TR, the cross-products and the associated correlation coefficients are shown in Table IV. All the correlation coefficients, whether between population means, salinity means or for individuals within classes, are negative. The correlations between population mean TS and TR are shown within salinity, since with only one degree of freedom between populations overall the correlation must be +1 or —1. There is remarkable similarity between the correlations in the experiments with and without salinity acclimation. The largest correlations are between the mean TS and TR of populations at each salinity (see Tables II and III for the means).

Indices of temperature tolerance

To express temperature tolerance as a single value, it seemed reasonable to combine TS and TR into an index. While TS and TR are related, as discussed

TABLE V

Indices of temperature tolerance of two populations of E. affinis
(Index = $30 + TS - TR$).

	Salinity ‰ (with acclimation)			
	3	9	12	15
March population	14.2	25.2	20.2	32.2
August population	21.0	28.4	31.3	41.7
	Salinity ‰ (without acclimation)			
	0-1		12	
March population	12.6		21.2	
August population	19.7		25.7	
	F values and significances			
	With acclimation		Without acclimation	
Between populations	21.4**		3.5†	
Between salinities	26.9**		5.5*	
3 vs. 9, 12, 15‰	54.4**			
9 vs. 12, 15‰	6.4*			
12 vs. 15‰	20.0**			
Interaction	1.3†		0.2†	

* $P < 0.05$

** $P < .0.01$

† Not significant

Standard deviations within subclass were 7.9 minutes (with acclimation) and 9.8 minutes (without acclimation)

above, they are not so closely related that inclusion of both in an index is redundant. Four alternative possibilities are listed; I Rank on TS, then on TR; II Sum the rankings, downward by TS and upward by TR; III $30 + TS - TR$, 30 added to keep index positive; IV $100 + (\overline{TR} \times TS) - (\overline{TS} \times TR)$, inversely weighting by means and adding 100 to keep index positive.

These indices can be compared on a limited scale using the data from the animals shocked twice in 15‰, as described earlier. An effective index of tolerance should result in somewhat similar rankings of the animals in both assays. Using each of the above indices in turn, the relative tolerances at each shock temperature are quite similar. Selecting the top 10 animals (of 20) within strain at each temperature shock we find that using index I or II, five animals are selected in both assays; and using III or IV, six are commonly selected. Thus there is no difference between the indices in this limited test of them. Index III seems preferable since it is a simple function of TS and TR. The variance of this index is also a simple function of the variances and the covariance between TS and TR. Since the covariance is subtracted and is negative, the index variance actually exceeds the sum of the variances of TS and TR. The more variable component

contributes more to the index variance and so to the discrimination, which is appropriate if the variance reflects innate differences between animals. One could of course find an index to maximize strain differences over all the assays, but even then it might apply only to the current data.

In a later experiment 12 animals were tested and then re-tested after a period of about one hour at room temperature. The top five animals were the same in both assays and the correlation (repeatability) between the two index III values was 0.83. Consequently, one might infer that the index is measuring an inherent property of the animal quite accurately. The repeatabilities for times to succumb and to recover were 0.62 and 0.75, respectively, both below 0.83. One of the 12 animals did not succumb on either occasion. The average index was slightly higher in the second assay, so shocking the animals did not appear to decrease their tolerance.

Using index III ($30 + TS - TR$), the analyses shown in Tables II and III were repeated and the results are in Table V. Trends already noted are now clearer. There is a systematic increase with salinity in tolerance of the August population. The distinction between populations in 12‰ is greater following salinity acclimation. The advantage of the index over either TS or TR alone is suggested by the greater proportion of total variances attributable to population differences in the assays following salinity acclimation (compare F values in Tables II and V). In the assay without acclimation, the distinction using TS alone is greater (Tables III and V).

The results of re-analysis of the data from 36° shock at 15‰ (acclimated) are not shown in Table V. The index values were 19.9 (March) and 32.0 (August) and the difference is significant. As with the other assays on acclimated animals, the proportion of variance removed by populations is greater with the index than with TS or TR alone. Thus in general the index seems to enhance ascertainment of thermal tolerance, at least between the two populations used in this study.

Lower temperature tolerances

Low temperature tolerances of the March and August populations were investigated to a limited extent, using a temperature shock at 2.5°C. At 3‰ salinity (acclimated), means of TS were 3.4 (March) and 2.6 (August) and of TR were 6.7 (March) and 4.7 (August). At 9‰, four of the ten March animals did not succumb. The average TS and TR of the remainder were 6.1 and 5 minutes, respectively. None of the August animals succumbed at 9‰. Thus there appear to be strain differences at 9‰ for lower tolerance. The difference between populations tested in 3‰ were not significant. Increasing salinity seems to widen the range of tolerance as well as increase the upper limits of temperature tolerance.

DISCUSSION

The experiments described clearly show how salinity modified the effects of temperature. The results agree with other cases reviewed by Kinne (1964) where resistance to temperature extremes increased with salinity. The classic work on this subject is that of McLeese (1956) with the lobster *Homarus americanus*. He found systematic increases in upper lethal temperature with increase

in salinity and also with increasing acclimation temperature and oxygen concentration. Other examples of the effects of salinity on temperature sensitivity are given by Schlieper in Remane and Schlieper (1971), including work by Ranade (1957) on the copepod *Tigriopus fulvus* showing a continuous rise in lethal temperatures as a function of salinity. Thus there seems to be quite general agreement on the enhancement of temperature tolerance by increasing salinity, at least in the range below stressful salinities.

The effect of salinity acclimation was more marked in the August population, resulting in a greater distinction between the populations following acclimation. Also there was a differential effect of increasing salinity resulting in increasing distinction between the populations at higher salinities. This suggests that since strain differences are enhanced, individual differences in tolerance may be enhanced at higher salinities. One way to determine this would be to select for tolerance at more than one salinity.

It should be emphasized that what has been shown by the data reported here is a repeatable, salinity-dependent difference in temperature tolerance between two populations of *Eurytemora*, which were collected originally in March and in August. I have not demonstrated that there is a difference in tolerance in winter and in summer. To establish such a difference the effects of other variables, including salinity, must first be removed. We have preliminary evidence that temperature acclimation has a significant influence on temperature tolerance.

The question of the nature of seasonal differences in temperature tolerance, if they exist, therefore remains open. The adaptive strategy of the species may well be a combination of genetic and physiological adjustment. I now discuss the curious discrepancy between our results on temperature tolerance and the distribution of *Eurytemora* in the wild.

As suggested by the title of the paper, the effect of salinity on temperature tolerance is not consistent with the observed distribution of *Eurytemora* in the Chesapeake Bay region. During the warm summer months it is confined to the upper fresher reaches of rivers around the bay. We collected samples at 28° C in nearly fresh water in August. This temperature is near the upper tolerance level, at least in laboratory culture—I could not culture *E. affinis* at 30° C, nor could Heinle (1969a). Yet we found thermal tolerance was greatest in 15‰, a salinity beyond the range in which the species is normally found at any season. So why should the general increase in salinity with rising average temperature result in a retreat of the species to areas of lower salinity? The inference is that there is no cause-effect relationship between salinity and the distribution of *E. affinis*, so other explanations must be invoked.

One factor contributing to the observed distribution might be increased predation in the summer and fall. This seems unlikely since Heinle (1970) found the density of *Eurytemora* was less affected by predation than that of *Acartia tonsa* (Dana). So unless there is selective predation, other species should be affected at least as much as *E. affinis*.

A more likely factor influencing the distribution of *Eurytemora* is competition, especially with the dominant summer species, *Acartia tonsa*. According to Heinle (1969a, Fig. 10) the growth rate and productivity of *E. affinis* is equal to that of *Acartia* at 12° C but only half as great at 25° C. Furthermore the density of *Acartia* may be closely related to phytoplankton production, whereas the density

of *E. affinis* is not, resulting in additional competitive advantage to *Acartia* in the summer (Heinle, personal communication).

I wish to thank Joanne Janyska for collecting the data, Frank Hanson for critically reading the manuscript and Donald Heinle for advice on rearing the cultures. The work was supported by NSF Grant GA 33628.

SUMMARY

1. Thermal tolerances of populations of *Eurytemora affinis* were measured using two basic methods, at various salinities with and without acclimation. Little distinction in tolerances was made using temperature of inactivation. A more useful assay was temperature shocking at 34.5° C, observing time to succumb (TS) and time to recover (TR) over a 30 minute period.

2. Using the shock-recovery assay, there were repeatable and significant differences in tolerance between populations collected in March and August and also between salinities. Average tolerances and differences between populations generally increased with salinity.

3. The distinction between populations in thermal tolerance was greater when the animals were osmotically acclimated for 24 hours.

4. A simple index of tolerance combining TS and TR was suggested. In most cases the proportional variance between populations, using the index, was increased over TS and TR.

5. The seasonal distribution of *E. affinis* is contrary to that expected from the thermal tolerance-salinity relationship reported here. The explanation offered is that other species, for example *Acartia tonsa*, are at a competitive advantage during the summer and fall because of faster growth rate and greater dependence on phytoplankton.

LITERATURE CITED

- BATTAGLIA, B., 1967. Genetic aspects of benthic ecology in brackish waters. Pages 574-577 in H. W. Lauff, Ed., *Estuaries*. American Association for the Advancement of Science Publication Number 83.
- HEINLE, D. R., 1969a. Temperature and zooplankton. *Chesapeake Sci.*, **10**: 186-209.
- HEINLE, D. R., 1969b. Culture of calanoid copepods in synthetic seawater. *J. Fish. Res. Board Can.*, **26**: 150-153.
- HEINLE, D. R., 1970. Population dynamics of exploited cultures of calanoid copepods. *Helgolander Wiss. Meeresunters.*, **20**: 360-372.
- KINNE, O., 1963. The effects of temperature and salinity on marine and brackish water animals. I. Temperature. *Oceanogr. Mar. Biol. Ann. Rev.*, **1**: 301-340.
- KINNE, O., 1964. The effects of temperature and salinity on marine and brackish water animals. II. Salinity and temperature salinity combinations. *Oceanogr. Mar. Biol. Ann. Rev.*, **2**: 281-339.
- KINNE, O., 1967. Physiology of estuarine organisms with special reference to salinity and temperature, general aspects. Pages 525-530 in H. W. Lauff, Ed., *Estuaries*. American Association for the Advancement of Science Publication Number 83.
- KINNE, O., 1970. *Marine Ecology*, Volume 1. Wiley-Interscience, 1774 pages.
- MCLEESE, D. W., 1956. Effects of temperature, salinity and oxygen on the survival of the American lobster. *J. Fish. Res. Board Can.*, **13**: 247-272.
- RANADE, M. R., 1957. Observations on the resistance of *Tigriopus fulvus* to changes in temperature and salinity. *J. Mar. Biol. Ass. U.K.*, **36**: 115-119.
- REMANE, A., AND C. SCHLIEFER, 1971. *Biology of Brackish Water*. John Wiley & Sons, Inc., New York, 372 pages.
- VERNBORG, W. B., AND F. J. VERNBERG, 1972. *Environmental Physiology of Marine Animals*. Springer-Verlag, New York, 346 pages.